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**(54) Title:** CHEESE STARTER MEDIA AND METHOD OF MAKING SAME

**(57) Abstract**

Low cost, readily dispersible, phage-resistant cheese starter media which include milk-derived nutrients (e.g., nonfat milk and whey) along with a minor proportion of preferably free or unbound lecithin. The media also may advantageously include sodium tetraphosphate which assists in the dispersion of whey solids. The media of the invention can be used at significantly lower levels as compared with nonfat dry milk solids (e.g., 7 percent versus 12 percent), while nevertheless obtaining essentially equivalent results in terms of culture growth and final culture properties. A method of producing the media is also disclosed, involving liquid preblending of phosphates and lecithin, followed by addition thereof to milk-derived nutrients and reaction of the phosphates to tie up free calcium ion. The final step involves drying of the mixture to yield a substantially homogeneous, reconstitutable powder. In other cases the phosphate-lecithin preblend can be dried for later addition to milk-derived nutrients to produce a final starter medium.

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CHEESE STARTER MEDIA AND METHOD OF MAKING SAME

5 This is a continuation-in-part of application Serial No. 06/483,508, filed April 11, 1983 and entitled "Cheese Starter Media."

Background of the Invention1. Field of the Invention

10 The present invention is broadly concerned with novel, low cost cheese starter media which can be used by cheese makers in the growth of bulk starter cultures, along with a unique method of producing the media. More particularly, it is concerned with such media which in preferred 15 forms include a minor amount of free or unbound lecithin, and/or minor quantities of sodium tetraphosphate for purposes of imparting a stable, homogeneous dispersion of the starter media ingredients; in its method aspects, the invention 20 involves preparation of a liquid preblend including phosphate anti-bacteriophage agent(s) and, preferably, lecithin, and mixing of the preblend with milk-derived nutrients (e.g., whey and nonfat milk), followed by drying to yield a smooth, 25 consistent, substantially uniform and homogeneous reconstitutable powder.

2. Description of the Prior Art

30 In the manufacture of natural cheese, milk in a cheese vat is inoculated with a minor amount (e.g. 2-4 percent) of a bulk starter providing the necessary culture of acid-forming 35 microorganisms used for the particular cheese being manufactured. For example, in the case of Italian cheeses such as mozzarella, it is the usual practice to employ Streptococcus thermo-



1        philus together with one or more lactobacilli such  
as Lactobacillus bulgaris. In the art, the strep-  
tococci are generally referred to by the short  
name of "coccus", while the lactobacilli are  
5        referred to as "rod" bacteria because of their  
appearance under microscopic examination.

10       The quantity and activity of cheese-  
making microorganisms can be critical to the  
overall outcome of the process and final cheese  
quality. Again referring to Italian cheese, it  
has been found that, in order to make acceptable  
cheese, the ratio of coccus to rod microorganisms  
in the starters should be from about 1:1 to 5:1,  
the most preferable level being about 2:1 to 3:1.  
15       If these ratio considerations are not met, the  
final Italian cheese product may be deficient in  
flavor or physical properties such as elasticity  
and "stringiness."

20       It is the universal practice among  
cheese makers to grow their bulk starters using  
relatively minor amounts of seed culture. In such  
techniques, the seed culture is inoculated into a  
starter medium and allowed to incubate therein so  
that the culture cells will multiply to produce  
25       the desired bulk starter for use in cheese making.  
Here again, the types of starter media and the  
techniques used during the incubation process can  
have a relatively critical outcome on the quality  
of the final bulk starter, and hence on the cheese  
30       ultimately produced. A dilute dispersion of  
nonfat milk (e.g., 12 percent solids level) in  
water has long been considered the starter medium  
of choice. However, use of nonfat milk in this  
context is a relatively expensive proposition, and  
35       therefore cheese makers have in the past sought to



1 use media of a less expensive nature which either  
5 eliminate nonfat milk entirely, or sharply limit  
10 its use by provision of substitute materials.

For example, U.S. Patent No. 3,852,158  
15 describes a starter media which includes milk-  
derived materials, a nitrogen source, and citrate  
anion. In preferred forms, the starter media  
described in this patent contain a major amount of  
sweet whey and a minor amount of nonfat dry milk  
20 solids.

U.S. Patent No. 3,998,700 describes  
25 starter media which include both acid and sweet  
whey solids together with nonfat dry milk solids.  
Finally, U.S. Patent No. 2,805,950 describes the  
use of whey for culturing bacterial microorganisms  
30 used in making a swiss cheese.

While a number of alternative starter  
35 media have thus been proposed in an attempt to  
provide an acceptable substitute for expensive  
nonfat milk, none of these media have given re-  
sults completely equivalent to that of the nonfat  
milk. In many cases, the alternative media do not  
provide the ideal environment for bacterial  
growth, or in the case of Italian cheese making,  
the final coccus to rod ratio obtained may be  
improper. Moreover, in those media which incor-  
porate relatively large quantities of whey, a  
problem arises by virtue of the phenomenon known  
as "whey out." Specifically, large amounts of  
whey in a starter medium can precipitate to the  
bottom of the starter tank and create severe  
handling problems. In fact, these problems can  
become so severe that some cheese makers simply  
refuse to use starter media containing substantial  
amounts of whey, even if growth characteristics of  
such media are satisfactory.



1                   U.S. Patent No. 3,041,248 to Hargrove  
describes the use of various phosphates for the  
control of bacteriophage, which are active against  
lactic acid bacteria. Indeed, the many starter  
5                   media presently available include various phos-  
phates for the purpose of combatting bacterio-  
phage. In this connection, it is known that the  
phosphates react with or tie up the readily avail-  
able calcium ion present in the media, and this in  
10                  turn prevents the bacteriophage from adsorbing  
onto the specific starter bacterium. In conven-  
tional practice, the phosphates are simply added  
directly to the remaining dry ingredients of a  
starter medium, followed by appropriate blending  
15                  and bagging. This conventional dry blending  
procedure presents a number of practical problems  
in the use of starter medium, however, particular-  
ly with respect to the phosphate content thereof.

20                  Specifically, the phosphates tend to be  
of irregular, grainy appearance and size in a dry  
condition, and therefore tend to settle out or  
stratify in the dry blended media. When the media  
are reconstituted in water, problems are presented  
not only from the standpoint of solubility (the  
25                  conventional media are sometimes difficult to  
disperse in water), but more important the phos-  
phates present may not completely react with free  
calcium ion. In order to ensure the most effec-  
tive use of the phosphate anti-bacteriophage  
30                  agents, it is desirable that the dry medium be  
smooth, uniform and substantially homogeneous; and  
this is particularly the case when it is borne in  
mind that the media may be used with radically  
different equipment and cheese-making practices  
35                  from manufacturer to manufacturer. Non-uniformity



1      inevitably means that in certain portions of the media the phosphate concentration is too low, while in other portions it is too high; and both of these conditions should be avoided.

5      In addition, when a typical dry blended powder medium is reconstituted in water, it is desirable to allow sufficient time for the phosphate to react with available calcium. Under normal cheese plant conditions, however, this reaction time should be minimized, and in some instances time constraints have forced cheese makers to employ a starter medium which has been insufficiently reacted; the result of this is that the problem of bacteriophage may not have been completely eliminated, and this in turn can have severe consequences in terms of cheese production.

10     In short, the irregular, non-uniform nature of many dry blended starter media compositions lead to a number of rather serious problems, most particularly with respect to the proper utilization of phosphate anti-bacteriophage agents present therein.

15     Accordingly, there is a heretofore unsatisfied need in the art for less expensive, alternative starter media, and a method of production thereof, which can be used in lieu of nonfat milk per se while giving essentially equivalent results in terms of culture growth and qualities, and which avoids practical difficulties such as "whey out" and problems stemming from non-uniformity.

#### Summary of the Invention

20     The problems outlined above are in large measure solved by the present invention which



1 provides greatly improved starter media for cheese  
making microorganisms. A principal advantage of  
the invention is the fact that dried media can be  
readily produced which are virtually homogeneous  
5 and give the appearance of a fine, talcum-like  
powder. The uniformity of the media of the invention  
facilitates reconstitution and dispersal  
thereof in water or other aqueous media and sub-  
stantially prevents differential phosphate concen-  
10 trations.

15 In terms of production methods, dried,  
reconstitutable bacteriophage-resistant starter  
media for cheese-making microorganisms are pro-  
duced by first providing a quantity of milk-  
derived nutrient such as whey, nonfat dry milk  
solids, or a combination of the foregoing. In the  
next step, a liquid preblend is prepared, sepa-  
rately from the first quantity of milk-derived  
nutrients, with the preblend having a phosphate  
20 anti-bacteriophage agent dispersed therein.  
Advantageously, this preblend also includes a  
quantity of lecithin along with appropriate miner-  
als. The liquid preblend is then added to the  
milk-derived nutrients to form a liquid mixture,  
25 and this mixture is then allowed to react for a  
period of, typically, 1-12 hours in order to  
permit the phosphates to react with available  
calcium ion in the mixture. In the final manu-  
facturing step, the reacted mixture is dried,  
30 usually using conventional spray drying tech-  
niques.

35 In preferred manufacturing procedures,  
the pH of the milk-derived nutrients is adjusted  
to a level of from about 6.0 to 7.5 prior to the  
addition of the liquid preblend. Further, the

1        milk-derived nutrients may be partially concentrated to a solids level of from about 25 to 50 percent, and the temperature thereof is advantageously adjusted to from about 35 to 60 degrees  
5        Fahrenheit, in order to facilitate dispersal of the preblend therein.

10       The preblend is normally prepared by heating a quantity of water to a temperature of from about 85 to 130 degrees Fahrenheit, followed by addition of the phosphate agent(s) such as mono and disodium phosphate and sodium tetraphosphate to the heated water, with agitation to achieve a substantially uniform dispersion. In preferred procedures, an amount of free or unbound lecithin  
15       is also added to the preblend mixture.

20       The final dried starter media in accordance with the invention are very uniform and homogeneous, and exhibit extremely desirable physical characteristics which greatly facilitate their use. As noted, such media in dried form broadly comprise a milk-derived nutrient and a minor amount of unbound lecithin. The milk-derived nutrients used in the preferred starter media advantageously comprise relatively modest amounts of nonfat milk, and major proportions of whey. Starter media in accordance with the invention which include substantial whey fractions are greatly improved by the addition of a minor amount of sodium tetraphosphate therein, which as noted is added with the other phosphates in the liquid preblend. This additive has been found to greatly assist in the aqueous dispersion of the whey, and largely eliminates the problem of "whey out."



1                   The addition of free or unbound lecithin  
to the starter media hereof has been found to give  
enhanced results in terms of culture growth and  
final bulk starter properties; indeed, the pre-  
5                   ferred media of the invention give essentially  
equivalent results, as compared with use of nonfat  
dry milk solids in aqueous dispersion. In fact,  
such equivalent results obtain through the use of  
10                  significantly smaller quantities of the present  
media, as compared with NFDM. Specifically, a 7  
percent solids dispersion of the preferred media  
of the invention gives virtually identical re-  
sults, as compared with a 12 percent solids dis-  
persion of NFDM.

15                  As used herein, the term "free" or  
"unbound" in conjunction with lecithin refers to  
lecithin in relatively purified form which is  
substantially free of chemical and/or physical  
bonding to other materials. Such free or unbound  
20                  lecithin is to be contrasted with, for example,  
lecithin which may be found in products such as  
fresh milk or buttermilk. In such context, the  
relatively minor lecithin content is believed to  
exist as a lipoprotein where the lecithin is in a  
25                  complex with the protein. Such association with  
other constituents may have the effect of altering  
the properties of the lecithin in the context of  
starter media; accordingly, use of the free or  
unbound lecithin as herein defined is preferred.

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1           The media of the invention also advantageously include minerals such as manganese chloride and ferrous ammonium sulphate, and a corn steep solids/whey solids stimulant.

5           Although in preferred forms a complete dried starter medium is provided in accordance with the invention, in other instances a dried preblend can be produced for addition to sweet whey or other milk-derived nutrients to produce a complete starter medium. Such a preblend would comprise a dried, substantially uniform powder having therein respective quantities of a phosphate anti-bacteriophage agent, lecithin and optionally NFDM, corn steep stimulant and minerals (e.g., ferrous ammonium sulfate and/or manganese chloride). The lecithin is preferably free or unbound as defined, and is advantageously present at a level of from about 0.1 to 0.8 percent by weight (most preferably, 0.5%).

20           Description of the Preferred Embodiments

25           In practice, one of the most preferred starter media of the invention is in the form of a dried composition which can be added to an aqueous system to give a liquid starter medium. This composition includes the following components:

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TABLE I

	<u>Ingredient</u>	<u>Initial Quantity<sup>1</sup></u>	<u>Parts by Wt. of Dried Composition (Dry Basis)</u>
5	Stimulant	700 lbs.	4.120
10	Nonfat dry milk solids	900 lbs.	5.290
15	Sodium tetra phosphate	800 lbs.	4.700
20	Disodium phosphate	550 lbs.	3.230
	Monosodium phosphate	800 lbs.	4.700
25	Manganese chloride	400 gr.	0.005
	Ferrous ammonium sulfate	400 gr.	0.005
30	Lecithin <sup>2</sup>	5 gallons	0.290
	Sweet liquid whey <sup>3</sup>	280,000 lbs.	77.660

25 <sup>1</sup> Total weight or quantity, including free water, of starting ingredients

2 <sup>2</sup> Lecithin in liquid form, 50% by wt. solids; or could be in the form of a dried powder

3 <sup>3</sup> May alternatively be derived by mixing 13,198 lbs. of dried whey with 266,802 lbs. of water

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1        The preferred dried powder media composition is made as follows. In the first step, 700 pounds of the stimulant (a dried mixture of corn steep solids and sweet whey solids described in detail below), along with 900 pounds of the nonfat dry milk solids are mixed with the 280,000 pounds of liquid sweet whey (either raw or pasteurized, normally pasteurized). After sufficient mixing to disperse the stimulant and milk solids, the mixture is neutralized by the addition of sodium hydroxide (50%) to a pH of 6.0-7.5 (preferably 7.0). The pH adjusted mixture is then thermally evaporated under vacuum conditions to a 25-50 percent solids level (preferably 41%), whereupon the mixture is cooled to 35-60 degree Fahrenheit (preferably 50° F.) and transferred to a final mixing tank.

20       A phosphate/minerals/lecithin premix is prepared separately from the mixture of stimulant, dried milk and whey. This premix is made by adding 375 gallons of water at 85-130 degrees Fahrenheit (preferably 96° F.) to a 1,000 gallon mixing tank. Next, 800 pounds of sodium tetra phosphate is added, followed by 800 pounds of monosodium phosphate and 550 pounds of disodium phosphate, all with constant agitation. The next step involves dissolving the 400 grams of ferrous ammonium sulfate and 400 grams of manganese chloride in a small amount of water, whereupon these minerals are added to the agitated mixture of water and phosphates. The 5 gallons of lecithin is then added to the premix tank, again with sufficient agitation to ensure homogeneity. Other phosphate anti-bacteriophage agents can of course be employed in place of the preferred phosphates



1 listed above, e.g., ammonium phosphates. Moreover, while dry granular phosphates can be added and dissolved, in other instances these can be made in plant by mixing the starting ingredients, 5 e.g., appropriate quantities of phosphoric acid and ammonia can be reacted to form the desired ammonium phosphates, and the products used directly without an intermediate drying step.

10 This premix is then added to the mixture of whey, stimulant and dried milk solids, whereupon the overall mixture is agitated for 1-12 hours, and preferably overnight, in order to assure that the phosphates react with available calcium ion in the mixture. The reacted mixture 15 is then spray dried to about 3-6 percent moisture (preferably 4%) to yield a substantially uniform and homogeneous, flowable, dried, powder-like material. Of course, if the medium is made in the plant where it is to be used, it may be advantageous not to dry the medium but rather to use the 20 same directly with appropriate dilution to achieve the desired solids content for the final liquid medium, i.e., from about 5 to 15 percent, and more preferably from about 7-11 percent.

25 In other cases, however, the phosphate/minerals/lecithin premix can be formulated alone and dried, whereupon it can later be added to whey or other milk-derived nutrients to yield a final medium. This procedure gives some of the advantages of the preferred techniques described above, 30 and avoids the expense of drying, storing and shipping of large quantities of whey and the other components of the complete medium.

35 The stimulant referred to above is made by taking 280,000 pounds of separated raw whey



1 from the cheese-making vat (such amount of whey  
being a separate quantity from that used in the  
starter media per se listed in Table I), and  
adjusting the pH thereof to a level of about 8.0  
5 with sodium hydroxide. The pH-adjusted whey is  
then evaporated to a 34 percent solids level, and  
cooled to 50 degrees Fahrenheit. The evaporated  
whey is then pumped into a tank containing 42,880  
pounds of commercially purchased corn steep liquor  
10 having a pH of 4.15. Such liquor is obtained from  
The Staley Corporation of Decatur, Illinois, and  
has a 50 percent solids level. This creates a  
mixture containing about 60 percent by weight corn  
steep solids and 40 percent by weight whey solids.  
15 The 60 percent-40 percent mixture is then agitated  
overnight, filtered and spray dried to about 4  
percent moisture. The resultant dried product is  
stored in 50 pound bags for subsequent use in the  
starter media. An alternate stimulant can be  
20 produced by replacing the corn steep liquor with  
liquid yeast extract or any other suitable liquid  
stimulant.

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1           The above described dried media composition can be used to obtain starter media for various types of microorganisms used in cheese making. For example, in order to obtain a starter media specifically designed for culturing micro-organisms used in making Italian cheeses such as mozzarella (referred to as "coccus" and "rod" microorganisms), the following procedure is followed. First, 3,950 pounds (476 gallons) of fresh water is pumped into the starter tank, and the water is heated to a level of 100-110 degrees Fahrenheit. Three hundred pounds of the dried starter medium of Table I above is next added to the water, with the aid of a powder horn. The pH of this mixture will be about  $6.6 \pm 0.1$ . The mixture is then heated to 190 degrees Fahrenheit and held for one hour at this temperature, whereupon the mixture is cooled to a temperature of 100-112 degrees Fahrenheit. At this point, the mixture is ready for inoculation with the desired coccus and rod cultures.

25           In another example, a dried composition very similar to that described above can be employed to prepare a culture medium for lactic cultures to be used in making American-type cheeses. From a compositional standpoint, the only difference involves the addition of a total of 2200 grams of ferrous sulfate. In this technique, 3,950 pounds (476 gallons) of fresh water is pumped into the starter tank, and is heated to a temperature of 100-130 degrees Fahrenheit. Three hundred pounds of the modified dried starter medium is next added to the heated water with the aid of a powder horn, giving a resultant pH of the mixture of approximately  $6.6 \pm 0.1$ . The mixture



1       is then heated to 190 degrees Fahrenheit, and held  
at this temperature for 1 hour, whereupon the  
medium is cooled to 74-80 degrees Fahrenheit.  
5       Here again, at this point in the procedure, the  
medium is ready for inoculation with the appropriate  
lactic cultures.

10       While in many instances the preparation of a complete, dried composition of the type described above is advantageous for reasons for ease of handling or the like, the invention is not so limited. That is to say, in appropriate circumstances a cheese manufacturer may wish to use whey derived directly from the cheese making operation, as opposed to having whey in the dried media composition. In such a case, one option would be to prepare a supplement mixture which can be added to liquid whey to produce a final liquid starter media. The stimulant, phosphates, minerals, nonfat dry milk and lecithin are premixed either by dry or wet blending, and are added to liquid whey. The pH of the mixture is then adjusted to a level of 6.6-6.7 through addition of, e.g., sodium hydroxide or ammonia, and the mixture is heated to 190 degrees Fahrenheit and held at that temperature for one hour. The mixture is then cooled to 100-112 degrees Fahrenheit, whereupon it is ready for inoculation with a desired culture.

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EXAMPLE 1

35       In this test a comparison was made between the two starter media, namely nonfat dry milk solids, and the most preferred lecithin-containing composition of the invention, in terms



1 of final coccus/rod ratios, bacterial counts and  
 5 the activities achieved using the respective  
 media.

10 The nonfat dry milk was reconstituted in  
 water at 12.0% solids in water, whereas the medium  
 hereof (Table I) was reconstituted in water at a  
 level of only 7 percent solids. Both media were  
 heat treated by heating to 190 degrees Fahrenheit  
 and holding at this temperature for one hour. The  
 15 media were then cooled to 102 degrees Fahrenheit  
 and inoculated with identical quantities (1%) of  
 coccus and rod cultures (Streptococcus thermo-  
philus and Lactobacillus bulgaris). The cultures  
 were then incubated in the respective media at 102  
 20 degrees Fahrenheit until the titratable acidity  
 exceeded 1.0; for the NFDM system this took about  
 5.5 hours, and for the medium of the invention  
 about 7 hours. At this point the cultures were  
 cooled to 40 degrees Fahrenheit. The two cultures  
 25 were then tested for titratable acidity, coccus/  
 rod ratio, pH and activity, all using conventional  
 techniques. The results of this test were:

TABLE II

Media	Final pH	Final Titratable Activity	Coccus/Rod Ratio	Total Bacterial Count	Total Activity
NFDM (12%)	4.20	1.02	4:1	$140 \times 10^7$	0.70
Inven- tion (7%)	4.35	1.02	4:1	$130 \times 10^7$	0.72

1                   As noted in Table II, the results using  
the costly NFDM are very similar to those obtained  
with the medium of the invention. This is very  
surprising in that a significantly greater quanti-  
5                   ty of NFDM was employed (12%) versus the invention  
(7%). At present day typical retail costs, the  
cost of using the medium of the invention to  
achieve equivalent results is on the order of only  
10                  50 percent of that of using NFDM as a starter  
medium.

15                  In another similar test, equal percent-  
age solids amounts of NFDM and the medium of the  
invention were tested (7% solids used in both  
cases). These tests were conducted in the same  
manner as heretofore described except that the 7  
percent solids NFDM media was incubated after  
inoculation until the pH reached about 4.25; this  
is the pH level where NFDM systems normally give a  
titratable acidity of greater than 1.0. That is  
20                  to say, if a 7% solids NFDM system is allowed to  
incubate to a 1.0% or greater titratable acidity,  
the pH would be abnormally low (e.g., around 3.7),  
and the bacteria would be injured or the coccus/  
rod ratio would be totally unacceptable.

25                  Accordingly, the incubation of the 7  
percent NFDM system was terminated at the normal  
pH achieved for full strength NFDM media, which  
took about 5 hours. On the other hand, the medium  
of the invention was incubated for a period of  
30                  about 7 hours until the titratable acidity ex-  
ceeded 1.0. The final results were:

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TABLE III

Media	Final pH	Final Titratable Activity	Coccus/Rod Ratio	Total Bacterial Count	Total Activity
NFDM (7%)	4.25	0.79	3:1	$68 \times 10^7$	0.58
Inven- tion (7%)	4.45	1.01	4:1	$120 \times 10^7$	0.71

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Thus, the low solids NFDM system proved deficient in titratable acidity, bacterial count, and activity, as compared with the invention. Moreover, on a cost basis the medium of the invention is far superior, even at equal solids levels.

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EXAMPLE 2

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In this example, a comparative test was made between a medium in accordance with the invention (Table I) prepared using the preblending and drying procedure of the invention, versus a compositionally identical medium made simply by dry blending all of the ingredients (the lecithin used in this case was also a dried powder). The separate media were then reconstituted in 60 degrees Fahrenheit water, with agitation as necessary to a 7 percent solids level, and cooked at 190 degrees Fahrenheit for one hour. The media were then cooled to 104 degrees Fahrenheit and inoculated with 0.1 ml. of milk grown coccus and rod culture.

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The inoculated media were then incubated until the pH thereof dropped to 4.8 (about 5 1/2 hours), whereupon the pH was raised to 6.2 by the



1       addition of 50 percent sodium hydroxide. The  
incubations were then allowed to continue until  
the titratable acidity in both cases was 1.10.  
With the liquid preblend, spray dried medium of  
5       the invention, this took a total of about 10 1/2  
hours, whereas with the dry blended medium a total  
incubation time of 11 1/4 hours was required.

10       At this point the media were cooled to  
55 degrees Fahrenheit and tested as set forth in  
certain entries of the following Table:

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25

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TABLE IV

COMPARISONS OF LIQUID PREBLENDING AND DRYING vs.  
 SIMPLE DRY BLENDING ON THE PHYSICAL AND CULTURE  
GROWTH-SUPPORTING PROPERTIES OF STARTER MEDIA

5	Comparison Number	Characteristics	Liquid Pre-blend	Spray Dried Medium	Dry Blended Medium
10	1.	Physical Appearance	Smooth and uniform-- similar to talcum powder	Gritty and irregular sizes of the various ingredients evident	
15	2.	Stratification of the media	None		Stratified-- Phosphates Tend to settle down in the powder
20	3.	Solubility	Instantly soluble even in the 60° F. water		5 minutes agitation required to solubilize in the 60° F. water
25	4.	Initial pH of the powdered medium when reconstituted	6.65		6.30
30	5.	Precipitation upon reconstitution and heating	None		Considerable precipitates settled to bottom
35	6.	Time to grow coccus and rod culture using the 1 step neutralization to arrive at final 1.10 titratable acidity	10 1/2 hrs.		11 1/4 hrs.

1	<u>Comparison Number</u>	<u>Characteristics</u>	<u>Liquid Pre-blend Spray Dried Medium</u>	<u>Dry Blended Medium</u>
	7.	Coccus/rod ratio after growth	3:1	1:1
5	8.	Total bacterial count per gram after culturing	$230 \times 10^7$	$200 \times 10^7$
10	9.	Activity measured in terms of titratable acidity	0.65	0.63
	10.	Smoothness of liquid medium after growth of culture	Smooth	Grainy--an appearance of buttermilk

15 The foregoing Table demonstrates the many advantages obtained through use of the liquid preblend-drying procedure for producing starter media. The smooth, uniform, essentially homogeneous nature of the media of the invention not only facilitates quick, easy handling, but also gives measurably enhanced results in terms of desirable coccus/rod ratios, bacterial counts and activities.

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### EXAMPLE 3

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This example sets forth another preferred media composition in accordance with the invention, which has been formulated to reduce the incubation time needed to reach a final titratable acidity level of about 1.10 from an average of 10 1/2 hours to about 7 1/2-8 1/2 hours.

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TABLE V

<u>Ingredient</u>	<u>Quantity Used</u>
1 Yeast extract	850 lbs.
Nonfat dry milk powder	1,000 lbs.
Disodium phosphate	1,479 lbs.
Monosodium phosphate	221 lbs.
Ferrous ammonium sulfate	17 lbs.
Manganese chloride	450 grams
2 Lecithin	2 1/2. gallons
Sweet liquid whey	Balance to yield 17,000 lbs. batch of dried medium

<sup>1</sup> Employed as a stimulant

<sup>2</sup> 50% solids liquid lecithin

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The medium of Table IV is made as outlined above in Example 1. In the first step the liquid whey, NFDM powder and yeast extract are mixed and ammonium hydroxide or anhydrous ammonia is added to adjust the pH to 7.0, followed by drying to about 40 percent solids and cooling to 50 degrees Fahrenheit. A liquid preblend of lecithin, the phosphates and the minerals is then made by dispersing these ingredients in 96 degrees Fahrenheit water as outlined in Example 1. The preblend is then added to the cooled nutrients, and the mixture is allowed to react overnight, and the pH is then adjusted as necessary using  $\text{NH}_4\text{OH}$  or anhydrous ammonia. Finally, the mixture is spray dried to about 4% moisture.

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EXAMPLE 4

5 A highly preferred lactic culture medium  
for growing cheddar cheese starter microorganisms  
is set forth below:

TABLE VI

	<u>Ingredient</u>	<u>Quantity Used</u>
10	<sup>1</sup> Corn steep solids	1,360 lbs.
	Yeast extract	425 lbs.
	Nonfat dry milk powder	1,000 lbs.
	Sodium tetrrophosphate	840 lbs.
	Disodium phosphate	435 lbs.
	Monosodium phosphate	1,275 lbs.
	Manganese chloride	250 grams
15	<sup>2</sup> Ferrous ammonium sulfate	10 lbs.
	Lecithin	2 1/2 gallons
	Sweet liquid whey	Balance to yield a 17,000 lb. batch of dried medium

20 <sup>1</sup> Stimulants

<sup>2</sup> 50% solids liquid lecithin

25 This medium is made as outlined above,  
wherein the corn steep solids, yeast extract, NFDM  
and whey are initially mixed, and a separate  
liquid preblend of the phosphates, minerals and  
lecithin in water is added thereto, followed by  
overnight reaction and drying.

30 The tests described in the foregoing  
examples were performed as follows:

1      pH    Hydrogen ion concentration was determined  
          using Beckman pH meter

5      Titratable Acidity

9 grams of the medium sample was titrated  
5      with 0.1 N sodium hydroxide using pheno-  
          phthalein as an indicator. A faint pink color  
          indicated the end point.

10     Coccus and Rod Ratio

A one in ten dilution of culture in water was  
10     smeared on a clean glass slide, stained with  
          methylene blue, and examined under a compound  
          microscope. The ratio was determined on the  
          basis of clump and individual counts.

15     Total Bacterial Count

15     The cultured samples were serially diluted in  
          sterile phosphate buffered water according to  
          the procedures outlined in the Standard  
          Methods for the examination of dairy products  
          and plated using tryptic soy agar fortified  
20     with 0.5 percent yeast extract. The plated  
          samples were incubated at 37 degrees Centi-  
          grade for 4 days. The counting and expres-  
          sion of the test results were done according  
          to the Standard Procedures.

25     Activity Test

25     2 grams of culture was inoculated into 100  
          ml. of sterile 10.0 reconstituted nonfat dry  
          milk. The nonfat dry milk was pretested for  
          the inhibitory compounds. The inoculated  
30     milk was incubated at 36 degrees Centigrade  
          for 45 minutes. At the end of incubation,  
          the temperature was gradually increased to 46  
          degrees Centigrade within a span of 30 min-  
          utes and it was thereafter maintained at that  
          temperature for a period of 1 hour. The

1 samples were then chilled to prevent any  
further acid development. Ten grams of the  
sample was carefully weighed into a 25 ml.  
beaker. Ten drops of indicator (pheno-  
5 phthalein) was added and the entire contents  
were titrated against 0.1 N sodium hydroxide  
until a faint pink color persisted for 15  
seconds. The results were expressed as  
percent titratable acidity.

10 Although the above described specific  
culture media are preferred in terms of composi-  
tion and method of preparation thereof, those  
skilled in the art will readily appreciate that  
15 the invention is not so limited. That is to say,  
in other forms of the invention, various substi-  
tute and/or additional materials may be employed,  
as opposed to those specifically recited in Tables  
I, V and VI, and moreover the amounts of use can  
20 be varied for the respective components. To give  
but a few examples, the preferred corn steep  
solids/sweet whey solids stimulant can be employed  
in an amount from about 1-10 percent by weight,  
and more preferably from about 2-6 percent by  
25 weight (dry basis). Other possible stimulants  
useful in this context include yeast extract,  
hydrolyzed vegetable proteins, pancreatic enzyme  
digests, and protease-treated milk. Such stimu-  
lants serve as non-specific growth rate enhancers  
30 which increase the rate of acid production and  
growth. Stimulants of this type have been known  
in the past, particularly in the context of start-  
er media which contain other materials besides  
strictly nonfat dry milk solids.

1           While the use of nonfat dry milk solids  
and whey is preferred in order to provide the  
requisite milk-derived nutrients for the overall  
media, other materials such as casein, the various  
5           caseinates, casein hydrolyzates, partially de-  
mineralized whey, and whey protein concentrates  
could also be used. In those instances where  
nonfat milk is employed, such should be present at  
10          a level of from about 1 to 10 percent by weight,  
and more preferably from about 4 to 6 percent by  
weight (dry basis). In like manner, when whey is  
employed, such should be present at a level of  
from about 50 to 90 percent by weight, and more  
preferably from about 70 to 80 percent by weight  
15          (dry basis).

As noted above, the use of sodium tetra-  
phosphate in the media of the invention is pre-  
ferred, particularly in those instances where a  
major proportion of whey is present. This serves  
20          to minimize the extent of so-called "whey-out" by  
aiding in the dispersion of the whey solids.  
Advantageously, the sodium tetraphosphate is  
present at a level of at least about 2 percent by  
weight, and more preferably from about 3 to 13  
25          percent by weight (dry basis).

The disodium phosphate and monosodium  
phosphate additives are employed in the preferred  
composition in order to inhibit bacteriophage.  
The monosodium phosphate should be used at a level  
30          of from about 2 to 8 percent by weight (dry ba-  
sis), whereas the disodium phosphate should be  
used at a level of about 3 to 13 percent by weight  
(dry basis). Moreover, the combination of sodium  
tetraphosphate with disodium phosphate and mono-  
35          sodium phosphate is particularly preferred inas-

1 much as this combination gives good whey dispersibility, bacteriophage protection, and a buffering capacity in the overall system.

5 While manganese chloride and ferrous ammonium sulfate have been employed in minor amounts as additive minerals, it will be readily seen that other minerals and levels of use can be employed. Particular minerals and optimum levels of use thereof are within the skill of the art.

10 The free or unbound lecithin forming a part of the preferred media of the invention should be present at a level of from about 0.05 to 25 percent by weight, and more preferably from about 0.20 to 1 percent by weight (dry basis).

15 The utility of free or unbound lecithin in the media of the invention is not fully understood, but it is believed possible that the presence of lecithin (a phospholipid) improves the cellular integrity of the cheese-making microorganisms and thus promotes growth. In addition, lecithin is known to be an emulsifier, and could assist in the transport of nutrients into the cellular structure of the microorganisms. However, it will be appreciated that the foregoing represents hypothesis, and there is no wish to be bound to any sort of theory of operability in connection with use of lecithin.

20 During use of the media in accordance with the invention, it is desirable that the final liquid medium have a solids content of from about 5 to 15 percent by weight and more preferably from about 7-12 percent by weight, and most preferably from about 7-8 percent by weight. Obviously, use of the smallest amount of solids is preferred for economic reasons.



1           A variety of culture-growing techniques  
can be employed with use of the media of the  
invention. As noted above, the traditional tech-  
nique of inoculating the medium with microorgan-  
isms at a starting pH in the range of, typically,  
5           6.0-6.5, followed by incubation until the medium  
exhibits a pH in the range of 4.0-4.5, gives  
excellent results. In addition, certain other pH  
modification techniques described in recent years  
10          (see, e.g., U.S. Patent No. 4,282,255) can be used  
to good effect in conjunction with the improved  
media of the invention.

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1      Claims

5            1. A method of making a dried, re-constitutable, bacteriophage-resistant starter medium for cheese-making microorganisms, said method comprising the steps of:

10            providing a first quantity of milk-derived nutrient;

              preparing, separately from said first quantity of milk-derived nutrient, a liquid preblend having a phosphate antibacteriophage agent dispersed therein;

15            adding said liquid preblend to said milk-derived nutrient to form a liquid mixture, and allowing said agent to react with available calcium ion in said mixture; and

              drying the reacted mixture.

20            2. The method of Claim 1, said nutrient comprising sweet whey.

              3. The method of Claim 1; said nutrient comprising nonfat milk.

25            4. The method of Claim 1, including the step of adjusting the pH of said first quantity of milk-derived nutrient to a level of from about 6.0 to 7.5.

30            5. The method of Claim 1, including the step of drying said first quantity of milk-derived nutrient to a level of from about 25 to 50 percent solids.

1                   6. The method of Claim 5, including  
the step of adjusting the temperature of the dried  
nutrient to a level of from about 35 to 60° F.

5                   7. The method of Claim 1, said pre-  
blend preparation step comprising the steps of:  
heating a quantity of water to a temperature  
of from about 85 to 130° F.; and  
10                 adding said agent to the heated water, and  
agitating the resultant mixture to  
substantially uniformly disperse the  
agent therein.

15                 8. The method of Claim 7, said agent  
being selected from the group consisting of mono-  
sodium phosphate, disodium phosphate, sodium  
tetraphosphate and mixtures thereof.

20                 9. The method of Claim 1, including  
the step of agitating the admixed preblend and  
milk-derived nutrient prior to said drying step.

25                 10. The method of Claim 1, including  
the step of allowing said agent to react for a  
period of from about 1 to 12 hours prior to said  
drying step.

30                 11. The method of Claim 1, said leci-  
thin being present at a level of from about 0.2 to  
1.0% by weight in the final dried medium.

35                 12. The method of Claim 1, there being  
from about 50 to 90% by weight whey solids and  
from about 1 to 10% by weight nonfat milk solids  
in the final dried medium.



1                   13. A dried, reconstitutable, bacteriophage-resistant starter medium made by the method of Claim 1.

5                   14. A preblend for addition to a milk-derived nutrient to produce a starter medium for cheese-making microorganisms, said preblend comprising a dried, substantially uniform powder having therein respective quantities of a phosphate anti-bacteriophage agent, lecithin, one or 10 more stimulants and one or more minerals.

15                  15. The preblend of Claim 14, said agent being selected from the group consisting of monosodium phosphate, disodium phosphate, sodium tetraphosphate and mixtures thereof.

20                  16. The preblend of Claim 14, said lecithin being unbound lecithin.

17. The preblend of Claim 14, said lecithin being present at a level of from about 0.1 to 0.8% by weight.

25                  18. A starter medium for cheese-making microorganisms comprising a dried powder including whey and a minor amount of sodium tetraphosphate therein for assisting in the aqueous dispersion of said whey.

30                  19. The medium of Claim 18, said sodium tetraphosphate being present at a level of from about 3 to 13% by weight on a dry basis.

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1                   20. The medium of Claim 18, including  
respective minor amounts of monosodium phosphate  
and disodium phosphate.

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# INTERNATIONAL SEARCH REPORT

PCT/US84/00540  
International Application No.

## 1. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) <sup>3</sup>

According to International Patent Classification (IPC) or to both National Classification and IPC  
Int. Cl. 3 C12N 1/20; A23C 21/00; 19/00; 21/02; 9/12  
U.S. Cl. 435/253; 426/583, 36, 41, 43

## II. FIELDS SEARCHED

Minimum Documentation Searched <sup>4</sup>

Classification System	Classification Symbols
U.S.	435/253; 426/580, 582, 583, 34, 36, 42, 43

Documentation Searched other than Minimum Documentation  
to the Extent that such Documents are Included in the Fields Searched <sup>5</sup>

## III. DOCUMENTS CONSIDERED TO BE RELEVANT <sup>14</sup>

Category <sup>6</sup>	Citation of Document, <sup>15</sup> with indication, where appropriate, of the relevant passages <sup>17</sup>	Relevant to Claim No. <sup>18</sup>
A, P	US, A, 4,402,986, published 06 September 1983 Sinkoff et al.	1-20
A	US, A, 3,354,049, published 21 November 1967 Christensen, V.W.	1-20
A	US, A, 3,192,124, published 29 June 1965, Kheshgi, S., see Col. 6, lines 23-24.	1-20
A	US, A, 4,115,199, published 19 September 1978, Porubcan et al. see Col. 3, lines 40-50.	1-20
A	US, A, 4,289,788, published 15 September 1981, Cajigas, S.D., See Col. 6, lines 53-60	14,16,17
A	US, A, 4,289,789, published 15 September 1981, Cajigas, S.D., See Col. 6, lines 4-11	14,16,17

- Special categories of cited documents: <sup>16</sup>
- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step
- "Y" document of particular relevance; the claimed invention can be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "A" document member of the same patent family

## IV. CERTIFICATION

Date of the Actual Completion of the International Search <sup>9</sup>

28 June 1984

Date of Mailing of this International Search Report <sup>2</sup>

02 JUL 1984

International Searching Authority <sup>1</sup>

ISA/US

Signature of Authorized Officer

David M. Naff

**FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET**

A ,P	US, A, 4,382,965, published 10 May 1983, Sandine et al.	1-20
A	US, A, 4,282,255, published 04 August 1981, Sandine et al.	1-20
A	US, A, 3,041,248, published 26 June 1962, Hargrove et al.	1-20
A	US, A, 4,020,185, published 26 April 1977, Andersen et al.	1-20

**V.  OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE <sup>10</sup>**

This International search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1.  Claim numbers \_\_\_\_\_, because they relate to subject matter<sup>11</sup> not required to be searched by this Authority, namely:

2.  Claim numbers \_\_\_\_\_, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful International search can be carried out<sup>12</sup>, specifically:

**VI.  OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING <sup>13</sup>**

This International Searching Authority found multiple inventions in this International application as follows:

1.  As all required additional search fees were timely paid by the applicant, this International search report covers all searchable claims of the international application.

2.  As only some of the required additional search fees were timely paid by the applicant, this International search report covers only those claims of the international application for which fees were paid, specifically claims:

3.  No required additional search fees were timely paid by the applicant. Consequently, this International search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4.  As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

**Remark on Protest**

- The additional search fees were accompanied by applicant's protest.
- No protest accompanied the payment of additional search fees.

## III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category <sup>16</sup>	Citation of Document, <sup>16</sup> with indication, where appropriate, of the relevant passages <sup>17</sup>	Relevant to Claim No <sup>18</sup>
A	US, A, 4,053,642, published 11 October 1977, Hup et al.	1-20
A	US, A, 3,998,700, published 21 December 1976, Reinbold et al.	1-20
A	US, A, 4,372,979, published 08 February 1983, Reinbold et al.	1-20
A	N, Journal of Dairy Science, Vol. 44, issued July-December 1961, Hargrove et al., Phosphate Heat Treatment of Milk To Prevent Bacteriophage Proliferation in Lactic Cultures, pages 1790-1810, see page 1800, 6th paragraph	1-20
A	N, Utah Science, issued Winter 1979, Richardson et al., USU Lactic Culture System, pages 94-99.	1-20